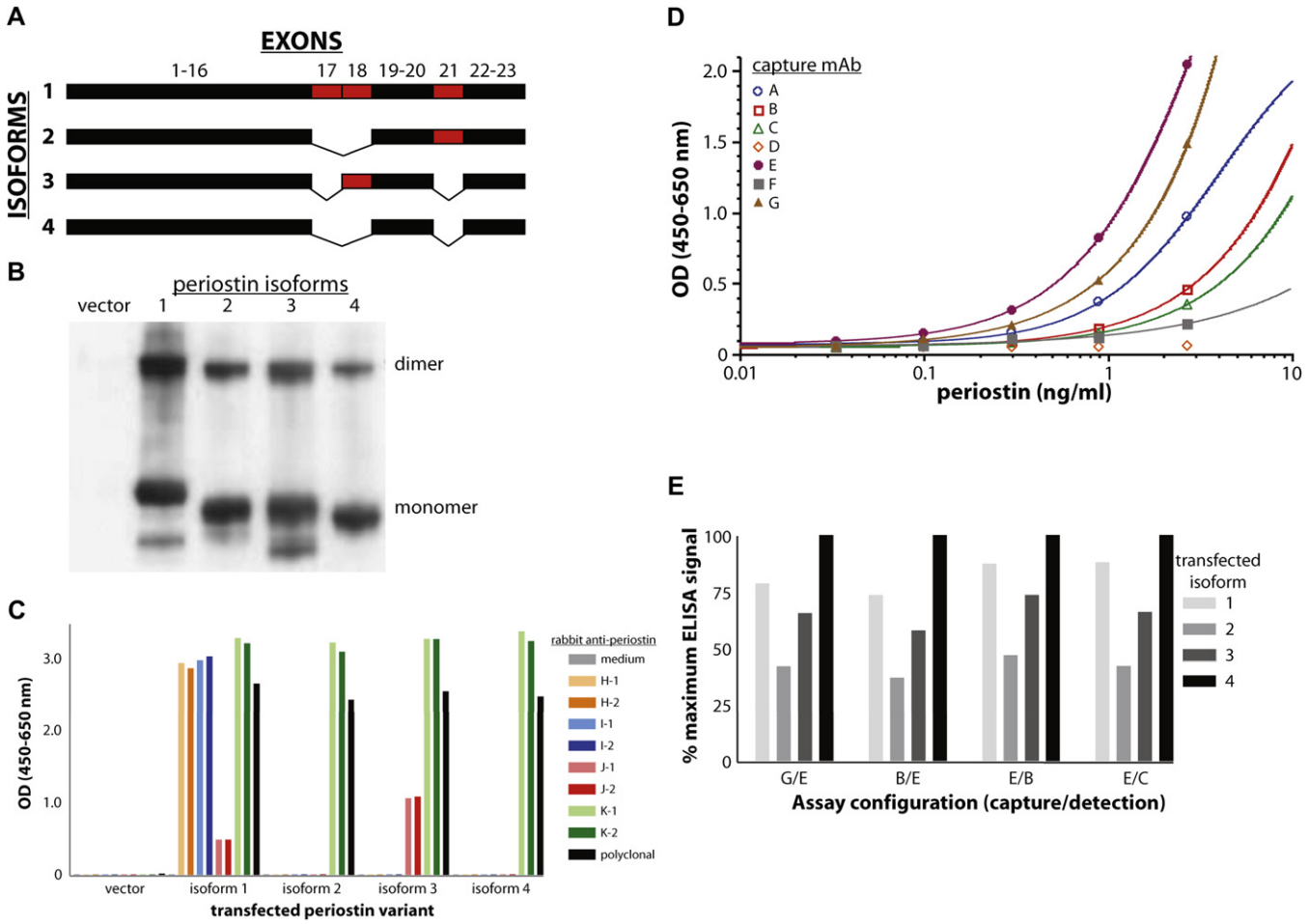

FIG E1. Development of a sandwich ELISA to detect serum and plasma periostin. **A,** Schematic diagram of exon use in alternatively spliced forms of human periostin. **B,** Immunoblot of cell-culture supernatants of HEK293 cells transfected with expression constructs encoding periostin isoforms 1 to 4 probed with rabbit polyclonal anti-periostin antibodies. Monomeric (approximately 90 kDa) and dimeric (approximately 180 kDa) forms of each isoform were detected as indicated. **C,** Detection of periostin isoforms in direct ELISA by rabbit monoclonal anti-periostin. Two subclones are shown for each parental clone, as indicated in the legend. **D,** Standard curves of the sandwich ELISAs with murine anti-periostin mAbs (capture) and rabbit monoclonal anti-periostin (clone K; detection). **E,** Detection of periostin isoforms by means of sandwich ELISA with murine mAb pairs. Note: differences in detection of different isoforms is due to varying levels of protein in supernatant; each mAb pair detected all 4 isoforms comparably.

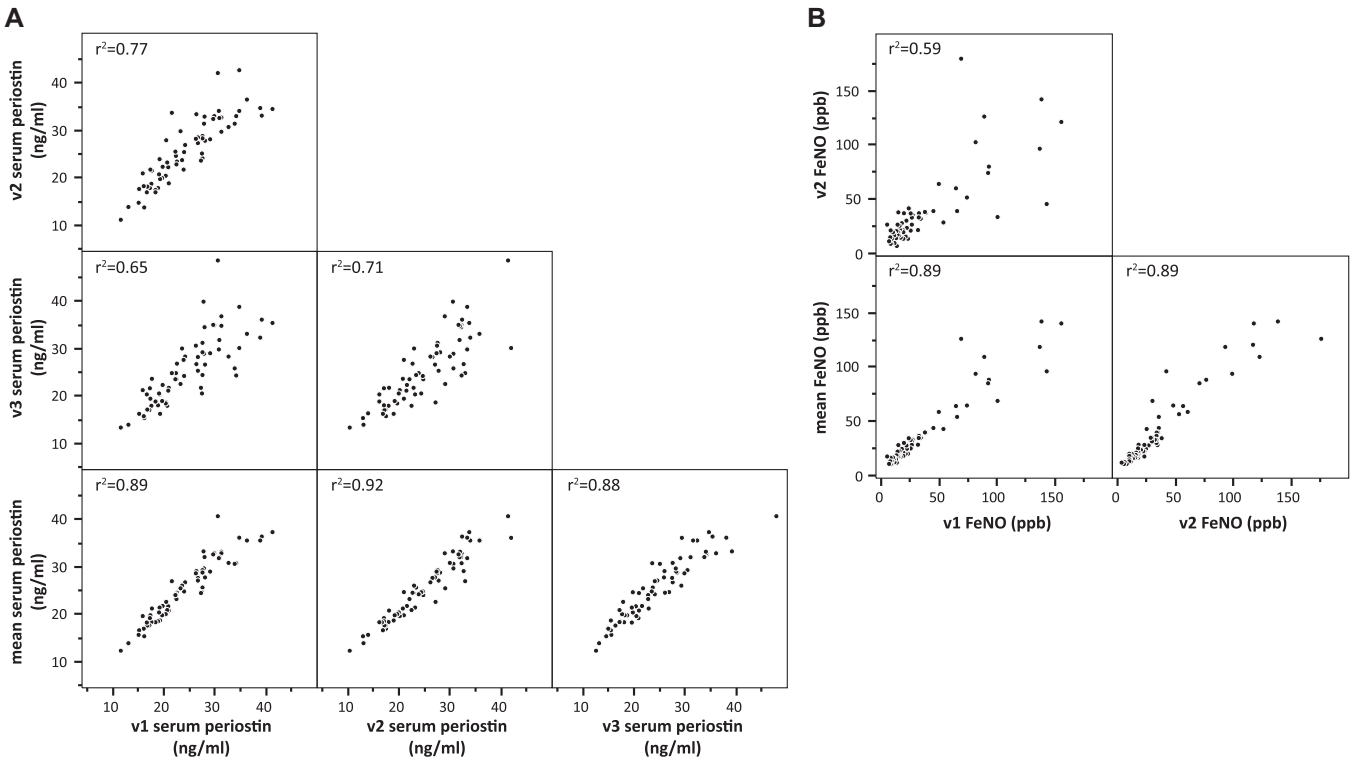

FIG E2. Intrasubject correlation between serum periostin and FENO levels across multiple visits in the BOBCAT cohort. **A,** Serum periostin levels at visits 1, 2, and 3 are highly correlated with each other and with the mean periostin level across all visits in BOBCAT. Accordingly, mean serum periostin levels across the 3 visits were used for analyses in Figs 2 and 3, Tables I and II, and Tables E4 and E5. **B,** FENO levels at visits 1 and 2 are highly correlated with each other and with the mean FENO level in BOBCAT. Accordingly, mean FENO level across the 2 visits was used for analyses in Fig 3, Tables I and II, and Tables E4 and E5.

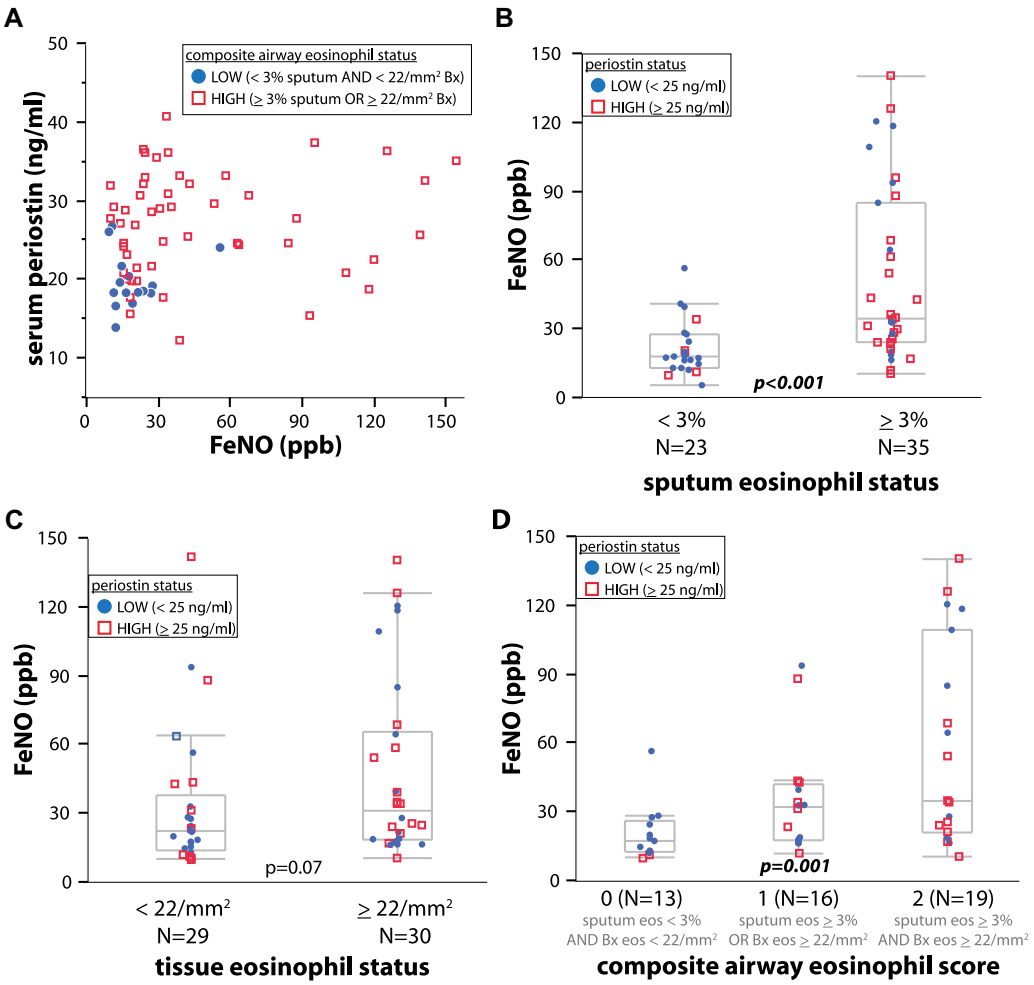

FIG E3. FENO levels differentiate patients with moderate-to-severe uncontrolled asthma receiving high-dose ICSs according to airway eosinophilic inflammation. **A,** Serum periostin and FENO levels are both increased in most subjects with increased sputum or tissue eosinophil numbers, but subsets of subjects have increases in only periostin or FENO levels. Most subjects lacking increased sputum and tissue eosinophil numbers exhibit low serum periostin and low FENO levels. **B,** Asthmatic patients with greater than 3% sputum eosinophils have significantly increased FENO levels compared with asthmatic patients with less than 3% sputum eosinophils ($P < .001$, Wilcoxon rank sum test). **C,** Asthmatic patients with greater than 22 eosinophils/mm^2 total bronchial tissue exhibit a trend toward increased FENO levels compared with asthmatic patients with less than 22 eosinophils/mm^2 total bronchial tissue ($P = .07$, Wilcoxon rank sum test). **D,** A composite airway eosinophil score in which 0 is defined as sputum eosinophils of less than 3% and tissue *(Bx)* eosinophil numbers of less than 22/mm^2, 1 is defined as either sputum eosinophils of greater than 3% or tissue eosinophil numbers of greater than 22/mm^2 (exclusive) and 2 is defined as both sputum eosinophils of greater than 3% and tissue eosinophil numbers of greater than 22/mm^2, demonstrating a strong positive progression for increasing FENO levels with increasing composite airway eosinophil scores ($P = .001$, logistic regression). Serum periostin status is indicated as in the legends.

TABLE E1. Selection of the murine mAb pair for periostin assay

	Biotinylated capture mAb					
Coat mAb	**A**	**B**	**C**	**D**	**E**	**G**
A	0.8	2.2	1.2	1.0	1.1	1.2
B	1.4	1.2	1.2	0.9	4.8	0.8
C	1.1	1.3	1.1	0.9	2.6	0.9
E	0.9	2.6	2.6	1.2	1.3	1.2
F	1.4	1.0	1.1	0.9	3.2	1.0
G	1.7	1.1	1.3	0.9	7.3	0.9

The signal/noise ratio at 33 pg/mL recombinant periostin is shown, as described in the Methods section in this article's Online Repository.

TABLE E2. Periostin assay qualification

Control	Mean (ng/mL)	SD (ng/mL)	%CV
Normal	29.47	1.57	5.32
High (spiked)	118.87	3.28	2.76

Details are provided in the Methods section in this article's Online Repository.

TABLE E3. Contingency table for cutoff values of serum periostin (n = 57) and FENO (n = 56) versus composite airway eosinophil status in BOBCAT

Test	Serum periostin >25 ng/mL		FENO >35 ppb	
Airway phenotype	**Eosinophil low**	**Eosinophil high**	**Eosinophil low**	**Eosinophil high**
Test result				
−	11	19	12	26
+	2	25	1	17
Sensitivity	0.57		0.40	
Specificity	0.85		0.92	
PPV	0.93		0.94	
NPV	0.37		0.32	
P value	.011		.042	

The *P* value is determined by using the Fisher exact test (2-tailed).
Eosinophil high, Sputum eosinophils >3% or biopsy eosinophils >22/mm^2; *Eosinophil low*, sputum eosinophils <3% and biopsy eosinophils <22/mm^2; *NPV*, negative predictive value; *PPV*, positive predictive value.

TABLE E4. Biomarker correlation matrix: Correlations between markers as continuous measures in BOBCAT

Biomarker	Comparator	r_S	*P* value
Periostin	BAL eosinophils (%)	0.17	.20
	Sputum eosinophils (%)	***0.46***	***<.001***
	Tissue eosinophils	**0.24**	**.06**
	Blood eosinophils	**0.22**	**.07**
	FENO	***0.27***	***.03***
	YKL-40	0.04	.77
Blood eosinophils	BAL eosinophils (%)	***0.31***	***.02***
	Sputum eosinophils (%)	***0.43***	***<.001***
	Tissue eosinophils	0.15	.25
FENO	BAL eosinophils (%)	0.21	.11
	Sputum eosinophils (%)	***0.46***	***<.001***
	Tissue eosinophils	***0.38***	***.003***
	Blood eosinophils	***0.43***	***<.001***
YKL-40	BAL eosinophils (%)	−0.07	.57
	Sputum eosinophils (%)	−0.05	.70
	Tissue eosinophils	−0.19	.15
	Blood eosinophils	−0.02	.90
	FENO	0.03	.81

Correlations with *P* values of less than .05 are highlighted in boldface italics.
Correlations with *P* values of between .05 and .10 are highlighted in boldface.
BAL, Bronchoalveolar lavage.

TABLE E5. Correlation matrix between expression levels of genes encoding biomarkers and T_H2 cytokines

	***POSTN*_210809_s_at**	***NOS2*_210037_s_at**	***CHI3L1*_209395_at**
IL13	***0.42 (.014)***	***0.37 (.029)***	−0.23 (.198)
IL5	***0.42 (.013)***	***0.34 (.048)***	−0.13 (.450)
*POSTN*_210809_s_at	–	***0.72 (<.001)***	−0.24 (.136)
*NOS2*_210037_s_at	–	–	***−0.34 (.027)***

Values are given as the Spearman rank correlation, with *P* value in parentheses. Correlations with *P* values of less than .05 are highlighted in boldface italics. IL-13 and IL-5 expression levels were determined from endobronchial biopsy specimens by using quantitative PCR. Expression of *POSTN* (*POSTN*_210809_s_at), *NOS2* (*NOS2*_210037_s_at), and *CHI3L1* (*CHI3L1*_209395_at), the gene encoding YKL-40, determined from bronchial epithelial microarray, as described in Woodruff et al,[E1] is shown.